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Comparison of Ozone and Chlorine Toxicity to the Developmental Stages of Striped Bass 'Morone saxatilis'

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16. ABSTRACT

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Comparison of Ozone and Chlorine Toxicity to the Developmental Stages of Striped Bass, *Morone saxatilis*[1]

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HALL, L. W. JR., D. T. BURTON, AND L. B. RICHARDSON. 1981. Comparison of ozone and chlorine toxicity to the developmental stages of striped bass, *Morone saxatilis*. Can. J. Fish. Aquat. Sci. 38: 752–757.

Toxicity of ozone-produced oxidants (OPO) to striped bass, *Morone saxatilis*, eggs, larvae, and fingerlings was determined under continuous-flow conditions. Eggs, tested in both fresh and estuarine water, were found to be significantly ($P < 0.001$) more sensitive to OPO in freshwater. The higher sensitivity found for striped bass eggs in freshwater suggests that ozone could have a more pronounced effect if discharged in freshwater industrial or municipal wastewaters located in the vicinity of a striped bass spawning area. The ozone data collected in this study were compared with previously published chlorine toxicity data for each striped bass life stage. The toxicity of chlorine and ozone was found to be similar with striped bass eggs and larvae in estuarine water. LC50's for fingerlings were 0.20 mg/L OPO at 6 h and 0.08 mg/L OPO at 96 h.

Key words: ozone, oxidants, chlorine, striped bass, *Morone saxatilis*; wastewaters

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La toxicité des oxydants dérivés de l'ozone(ODO) pour le bar d'Amérique, *Morone saxatilis*, ses oeufs, ses larves et ses alevins a été déterminée dans des conditions de débit continu. Lors d'essais avec de l'eau douce et de l'eau d'estuaire, on a constaté que les oeufs étaient nettement plus sensibles ($P < 0,001$) aux ODO dans l'eau douce. Ce fait donne à penser que l'ozone aurait un effet très puissant s'il était présent dans les eaux usées industrielles ou municipales déversées dans un cours d'eau à proximité d'une frayère de bar. Les données sur l'ozone recueillies au cours de l'étude ont été comparées à des données sur la toxicité du chlore pour le bar à chaque étape de sa vie, qui avaient été publiées auparavant. On a constaté que le chlore et l'ozone avaient une toxicité similaire dans les eaux d'estuaire pour les oeufs et les larves du bar. Les CL50 des ODO pour les alevins se chiffraient après 6 h et 96 h respectivement, à 0,20 et 0,08 mg/L.

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IN recent years chlorine has been widely used for disinfection of municipal and industrial wastewaters (Jolley et al. 1978). Although this biocide and its by-products are an effective disinfectant, its toxic effects on aquatic biota (Opresko and Hannon 1979; Hall et al. 1981b) and possible carcinogenicity to man (Jolley 1973) have caused concern. Several strong oxidizing agents such as bromine, bromine chloride, chlorine dioxide, hydrogen peroxide, iodine, and ozone have been suggested as alternatives to chlorine (Burton and Liden 1978). Ozone appears to be one of the most viable alternatives to chlorine for water and wastewater treatment (Evans 1972). The advantages of using ozone in place of chlorine in freshwater systems are (1) inorganic salt content is not increased after reaction; (2) taste or color of treated water is not affected; (3) it is a more powerful disinfectant; (4) undesirable residual effects may be eliminated; (5) it rapidly decomposes in water, and (6) it is generated on site thus reducing storage problems and assuring supply at a known price (Marine Research Inc. 1976).

Ozone toxicity to freshwater fish has been documented by various investigators (Frese 1974; Arthur and Mount 1975; Arthur et al. 1975; Rosenlund 1975; Le Paulone 1975; Wedemeyer et al. 1979; Asbury and Coler 1980); however, investigations on estuarine fish are limited (Toner and Brooks 1975; Block et al. 1978). This study was designed to (1) evaluate the effects of ozone on striped bass, *Morone saxatilis*, eggs, larvae, and fingerlings; (2) compare the toxicity of ozone with previously published chlorine toxicity data for

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each striped bass developmental stage, and (3) compare the toxicity of ozone to eggs in fresh and estuarine water. Striped bass were selected for this study because they are anadromous fish with spawning grounds sometimes located in the vicinity of industrial and municipal facilities which may discharge large amounts of biocides.

Materials and Methods

General Procedures

Water-hardened eggs of fertilized striped bass, *M. saxatilis*, were obtained from Brookneal Striped Bass Hatchery (freshwater) in Brookneal, Virginia, and transferred to the laboratory in sealed plastic bags with oxygen-saturated water at ~18°C. Eggs were maintained in both estuarine (3.5‰) and freshwater in 12-L hatchery jars. Larvae were reared in estuarine water from the egg stock to a total length of ~20 mm in hatchery trays at densities < 1 g/L. Larvae were fed natural plankton introduced via the estuarine system and supplemented with high densities of brine shrimp, *Artemia salina*. Fingerlings (~13 cm total length), obtained from a hatchery operation at Ichthyological Associates Incorporated in Middletown, Delaware, were acclimated for 14 d before testing. These organisms were fed precooked commercial shrimp twice daily during the acclimation period. The range of various water quality parameters during each study is listed in Table 1. Photoperiods for the acclimation and testing of all life stages were maintained at natural light (L)–dark (D) conditions which were ~12 h L:12 h D for eggs and larvae and 14 h L:10 h D for fingerlings.

Experimental Procedures

The water used in these studies was deep well water and estuarine water pumped into the laboratory from the Patuxent River, Maryland (~33 km from its outlet at Chesapeake Bay).

The effects of ozone-produced oxidants (OPO) to 12-h-old striped bass eggs were evaluated by continuously exposing this life stage to 0.00, 0.005, 0.01, 0.05, and 0.10 mg/L OPO (as equivalent total residual chlorine: TRC). Eggs in both fresh and estuarine water (Table 1) were exposed to OPO concentrations for periods of 12 and 30 h; hatch occurred when eggs were ~48 h old. Eggs were tested in both fresh and estuarine water because striped bass typically spawn in freshwater while the developing eggs and prolarvae are exposed to increasing salinities as they are transported down the estuary. All eggs were tested in cylindrical PVC test chambers (volume 206 mL) fitted with 350-μm nylon mesh placed in 7-L hatchery trays. Three sets of 30 eggs each were exposed to each test concentration. Mortality observations were recorded at the end of each exposure period. The criteria for death were a change from a normal transparent color to an opaque color and/or failure to hatch.

Striped bass larvae were subjected to continuous OPO concentrations of 0.00, 0.01, 0.05, 0.10, 0.15, 0.20, and 0.30 mg/L for periods up to 96 h. Forty larvae were tested at each concentration in 7-L hatchery trays. Mortality observations, water quality, and OPO concentrations were recorded at 6, 12, 24, 36, 48, 60, 72, 84, and 96 h. The criteria used for death of the larvae were cessation of opercular movement and/or lack of movement in response to a mild mechanical stimulus.

Table 1. Ranges of water quality during the striped bass egg, larvae, and fingerling studies. Egg studies were conducted in both estuarine and freshwater.

Life stage	Temp. (°C)	Salinity (‰)	pH	Dissolved oxygen (mg/L)
Eggs	17.4–17.5	3.5–3.6	7.7–7.8	6.9–9.0
Eggs	18.9–19.0	0	7.5–7.6	6.0–8.5
Larvae	22.8–23.1	11.1–11.3	7.6–7.7	6.0–9.5
Fingerlings	25.0–25.3	9.5–10.2	7.0–7.4	6.3–9.6

Ozone-produced oxidant effects on striped bass fingerlings were assessed by continuously exposing test organisms to OPO concentrations of 0.00, 0.01, 0.05, 0.075, 0.10, 0.20, 0.30, 0.40, and 0.50 mg/L for periods up to 96 h. Twenty organisms were tested at each concentration in 57-L glass aquaria. Mortality observations, water quality data, and OPO concentrations were recorded at 6, 12, 24, 48, 60, 72, and 96 h. Death criteria used for fingerlings were similar to those used for larvae.

Ozone gas was produced with an electrical discharge type ozonator (model T-816; Welsbach Ozone System Corp., Philadelphia, PA) using dry aviator oxygen. Ozonated oxygen (~4% O_3 by volume) was bubbled into the base of an 11.5-L polycarbonate counter current contact column (2.75 m height, 7 cm diameter) at 1.5 L/min through a 3-cm-fritted glass disc. Filtered Patuxent River water was pumped into the top of the column; ozonated water was drawn from the lower part of the column and fed through glass rotometers to head tanks. Filtered water and appropriate OPO concentrations were mixed in the head tanks to form the various study concentrations and delivered in continuous flow at 4 L/min through 13 mm PVC tubing to the vessels in which the tests were conducted.

Ozone-produced oxidant concentrations (±0.002 mg/L) were measured amperometrically during exposure as equivalent total residual chlorine (American Public Health Association, American Water Works Association and Water Pollution Control Federation 1976) every 6 h during the study using a PAR polarographic analyzer (model No. 174A; Princeton Applied Research Corp., Princeton, NJ). Other water quality data were also recorded at this time interval (See Table 1).

Statistical Procedures

The toxicity data for all striped bass life stages are presented in Table 2. Analysis of these data involved estimation of LC50 values, derivation of percent mortality predictive equations (Table 3), development of percent mortality response surfaces for larvae and fingerling (Fig. 1 and 2), and comparisons of sensitivity between striped bass eggs in fresh and estuarine water (Table 4). LC50 values for freshwater eggs (probit LC50's could not be calculated for estuarine eggs), estuarine larvae, and fingerlings were estimated by the

TABLE 2. Toxicity data for striped bass eggs, larvae, and fingerlings. The cumulative percent mortality is reported for each OPO concentration, although replicates were used with the eggs.

Life stage	Exposure period (h)	Cumulative percent mortality at OPO concentrations (mg/L)									
		0.00	0.005	0.010	0.050	0.075	0.100	0.15	0.20	0.30	0.40	0.50
Freshwater eggs	12	3.3	5.6	15.6	50.0	a	70.0	a	a	a	a	a
	30	6.7	5.6	25.6	43.3	a	80.0	a	a	a	a	a
Estuarine eggs	12	7.8	4.4	13.3	1.1	a	23.3	a	a	a	a	a
	30	3.3	3.3	5.6	6.7	a	8.9	a	a	a	a	a
Estuarine larvae	6	5.0	a	2.5	2.5	a	2.5	100.0	100.0	100.0	a	a
	12	5.0	a	2.5	2.5	a	5.0	100.0	100.0	100.0	a	a
	24	5.0	a	2.5	2.5	a	22.5	100.0	100.0	100.0	a	a
	36	5.0	a	2.5	2.5	a	42.5	100.0	100.0	100.0	a	a
	48	5.0	a	2.5	5.0	a	50.0	100.0	100.0	100.0	a	a
	60	5.0	a	2.5	5.0	a	50.0	100.0	100.0	100.0	a	a
	72	7.5	a	2.5	5.0	a	70.0	100.0	100.0	100.0	a	a
	84	7.5	a	2.5	5.0	a	70.0	100.0	100.0	100.0	a	a
	96	7.5	a	2.5	5.0	a	72.5	100.0	100.0	100.0	a	a
Estuarine fingerlings	6	0.0	a	0.0	0.0	0.0	10.0	a	70.0	95.0	100.0	100.0
	12	10.0	a	0.0	0.0	0.0	35.0	a	90.0	100.0	100.0	100.0
	24	15.0	a	0.0	0.0	60.0	55.0	a	100.0	100.0	100.0	100.0
	48	15.0	a	0.0	0.0	60.0	70.0	a	100.0	100.0	100.0	100.0
	60	15.0	a	0.0	0.0	60.0	75.0	a	100.0	100.0	100.0	100.0
	72	15.0	a	0.0	0.0	60.0	75.0	a	100.0	100.0	100.0	100.0
	96	15.0	a	0.0	0.0	60.0	75.0	a	100.0	100.0	100.0	100.0

[a] Data were not taken at these concentrations.

methods of Finney (1971) using the probit procedures of SAS (Statistical Analysis System) (SAS Institute 1979) to compare previously published chlorine data with our ozone data. Predictive mortality models for each life stage and water type were derived using the stepwise procedures of SAS (SAS Institute 1979). The following linear model was used in the procedures:

$$\text{Arcsine } [(r/n)^{0.5}] = A + B \text{ concn.} + C \text{ time} + D \text{ dose} + E \text{ concn.}^2 + F \text{ time}^2 + G \text{ dose} \times \text{time} + H \text{ dose} \times \text{concn.} + I \text{ Log (concn.)} + J \text{ Log (time)} + E$$

where

concn. = concentration of OPO (mg/L),
time = exposure time (h),
dose = concentration × time,
r = response in a given experiment,
n = sample size in a given experiment,
A–J = coefficients estimated by least squares procedures.

The important prediction variables were selected by the stepwise procedure and are presented along with their estimated coefficients in the equations in Table 3. Analysis of variance (ANOVA) (Table 4) was used to compare the models between eggs in each type of water (Sokal and Rohlf 1969).

TABLE 3. Predictive percent mortality equations and respective ANOVA data for each life stage. Abbreviations used in the equations are p = percent mortality; c = concentration of OPO (mg/L), and t = exposure time (h). The natural logarithm was used in these equations.

Source	df	Sum of squares	Mean square	F value	Significance
(1) *Estuarine eggs*: $p = [\sin(0.20 + 13.15c^2 t)]^2$					
Regression	1	0.08	0.08	2.43	0.13
Error	28	0.90	0.03		
Total	29	0.97			
(2) *Freshwater eggs*: $p = [\sin(0.22 - 102.73c + 116.66 \log(c + 1))]^2$					
Regression	2	3.19	1.60	80.42	0.0001
Error	27	0.54	0.02		
Total	29	3.73			
(3) *Larvae*: $p = [\sin(0.005 + 1263.60c - 502.50c^2 + 0.56 \log(t) - 1267.38 \log(c + 1))]^2$					
Regression	4	24.40	6.10	210.32	0.0001
Error	58	1.76	0.03		
Total	62	26.15			
(4) *Fingerlings*: $p = [\sin(-0.36 - 7.50c^2 + 0.12 \log(t) + 8.33 \log(c + 1))]^2$					
Regression	3	19.87	6.62	110.28	0.0001
Error	50	3.00	0.06		
Total	53	22.88			

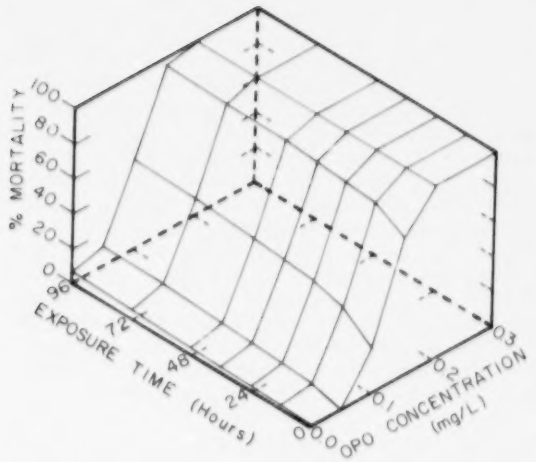

FIG. 1. Three-dimensional response surface of percent mortality for striped bass larvae exposed to OPO concentrations at various exposure times for periods up to 96 h.

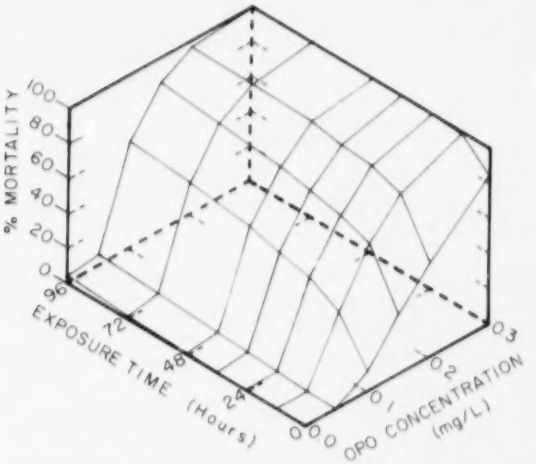

FIG. 2. Three-dimensional response surface of percent mortality for striped bass fingerlings exposed to OPO concentrations at various exposure times for periods up to 96 h.

Results and Discussion

EGGS

Predictive percent mortality models for estuarine and freshwater striped bass eggs are shown in Table 3. In both equations OPO concentration was an important factor contributing to mortality; exposure time was not significant. Eggs tested in estuarine water had a 12- to 30-h LC50 value of 0.21 mg/L OPO when calcualted by predictive equation (1) in Table 3. Eggs were found to be significantly ($P < 0.001$) more sensitive after 12- and 30-h exposures in freshwater than in estuarine water (Table 4). An LC50 value of 0.06 mg/L OPO (95% fiducial limits, 0.03-0.12) was found at both exposure times in freshwater using the probit procedure. A similar LC50 of 0.05 mg/L OPO was calculated from equation (2) in Table 3. The difference in toxicity of ozone in freshwater and estuarine water is probably caused by different residuals that remain after ozonation. Ozone reacts quickly in estuarine water to form several oxidant species (e.g. bromo compounds) which appear to be less toxic than ozone in freshwater which tends to remain longer in the more reactive molecular phase (Kosak-Channing and Helz 1979).

TABLE 4. A comparison of the sensitivity of freshwater and estuarine eggs to OPO using analysis of variance (ANOVA).

Source	df	Sum of squares	Mean square	F value	Significance
Water type	4	2.73	0.68	26.53	0.001
Error	52	1.34	0.03		
Total	56	4.07			

Delayed hatching of eggs was observed at 0.05 and 0.10 mg/L OPO in estuarine water. While in freshwater ~70% of the eggs hatched at the expected time (=48 h after fertilization) at 0.05 and 0.10 mg/L OPO; in estuarine water the hatch was 100% at 0.0 mg/L OPO, 100% at 0.005 mg/L OPO, 73% at 0.01 mg/L OPO, 8% at 0.05 mg/L OPO, and 7% at 0.10 mg/L OPO. It should be noted that in many cases where delayed hatch occurred, embryos were still alive within the chorion. No explanation for this delayed hatching can be given at the present time.

Middaugh et al. (1977) reported 0, 3.5, and 23% hatch for estuarine striped bass eggs continuously exposed for ~40 h to 0.21, 0.07, and 0.01 mg/L TRC, respectively. In our investigation we found 7% hatch for eggs in estuarine water exposed to 0.10 mg/L OPO for approximately the same period of time. It appears in each study that TRC and OPO have a similar effect on percent hatch.

Previous investigators have reported 24- and 48-h LC50's of 0.36 and 0.20–0.22 mg/L TRC, respectively, for striped bass eggs in estuarine water (Morgan and Prince 1977). The 12- to 30-h LC50 value of 0.21 mg/L OPO found in our study is lower than the 24-h LC50 value of Morgan and Prince (1977) but is similar to their 48-h LC50. Morgan and Prince (1978) did find that TRC concentrations of 0.05 mg/L had no significant effect on the percent hatch for this species; however, percent hatch for eggs in estuarine water was affected at 0.05 mg/L OPO in this study.

Burton et al. (1979a, b) conducted chlorine (0.00, 0.15, and 0.30 mg/L TRC), ΔT (2, 6, and 10°C), and exposure duration (0.08, 2, and 4 h) interaction studies with striped bass eggs in estuarine water. These investigators found that TRC and exposure duration were predominant factors contributing to mortality in the test organisms. In a univariate chlorine study, Hall et al. (1981a) found less than 6% mortality for striped bass eggs in estuarine water exposed to TRC concentrations of 0.06 mg/L for 12 h and 100% mortality for 36-h exposures at the same concentration. Exposure time was not found to be an important variable in the present study for

eggs in either estuarine or freshwater (see equations (1) and (2) in Table 3). The explanation for the differences in effect of exposure time is not clear; however, it may be related to different oxidant species or water quality conditions present during the studies.

Comparisons of data collected in this study with previously published data indicate that the effects of ozone on estuarine striped bass eggs is similar to that of chlorine. Freshwater chlorine toxicity data on eggs were not available; therefore, comparisons with ozone in this type of water were not possible. The higher sensitivity found for eggs in freshwater suggests that ozone could have a more pronounced effect if discharged in fresh industrial or municipal wastewaters located near a striped bass spawning area.

Larvae and Fingerlings

Predictive percent mortality equations and response surfaces for striped bass larvae and fingerlings in estuarine water are shown in Table 3 and Fig. 1 and 2, respectively. Ozone-produced oxidant concentration and exposure time contributed to mortality in both life stages. The LC50 values for the larvae ranged from 0.15 mg/L OPO (95% fiducial limits, undefined) at 6 h to 0.08 mg/L OPO (95% fiducial limits, 0.05–0.11) at 96 h. LC50's for fingerlings were 0.20 mg/L OPO (95% fiducial limits 0.13–0.27) at 6 h and 0.08 mg/L OPO (95% fiducial limits, 0.05–0.13) at 96 h. Mortality increased over time but tended to stabilize at the longer exposure periods for both life stages.

Middaugh et al. (1977) reported 48-h LC50 values of 0.04 and 0.07 mg/L TRC for 2- and 12-d-old striped bass larvae. In our investigation we found a 48-h LC50 of 0.09 mg/L OPO (95% fiducial limits, 0.07–0.12) for this developmental stage. Comparison of data from these two studies shows that larvae exhibit a similar response to both biocides.

Morgan and Prince (1977) reported 24-h LC50's of 0.20 and 0.19 mg/L TRC for 24- and 70-h-old estuarine striped bass larvae, respectively. The 24-h LC50 for larvae in our study was 0.11 mg/L OPO (95% fiducial limits, 0.04–0.20). Since our 95% fiducial limits overlapped the LC50's found by Morgan and Prince, it appears that the effects from each oxidant are similar.

Chlorine (0.00, 0.15, and 0.30 mg/L TRC), ΔT (2, 6, and 10°C), and exposure duration (0.08, 2, and 4 h) interaction studies have been conducted by Burton et al. (1979a, b) with 24-h-old estuarine striped bass prolarvae. Total residual chlorine, ΔT, and exposure time were all found to be important factors causing mortality to this life stage. Precise comparisons between the Burton et al. studies and the present investigation are difficult because of different testing procedures. However, Burton et al. found 100% mortality after 4 h of exposure at a concentration of 0.15 mg/L TRC and a ΔT of 0°C while in the present study we found a 6-h LC50 of ~0.15 mg/L OPO (95% fiducial limits, undefined). The difference in mortality response for the larvae in each study was probably due to differences in the ages of test organisms.

Previous investigations in our laboratory have shown that ~75% mortality occurred with estuarine striped bass larvae (7–9 mm) subjected to 0.06 mg/L TRC for 96 h (Hall et al. 1981a). In the present study we found a 96-h LC50 of 0.08 mg/L OPO (95% fiducial limits, 0.05–0.11) for striped bass larvae. A comparison between these studies suggess that larvae may be slightly more sensitive to TRC than OPO; however, this difference does not appear to be very large.

A comparison between data collected in this study and the published literature indicates that the toxicities of ozone and chlorine are generally similar for striped bass larvae in estuarine water. A comparison for fingerlings was not possible due to lack of chlorine data. In previous studies, Helz et al. (1978) reported similar bromoform yields from ozone and chlorine in estuarine water indicating that the reaction pathways and mechanisms may be similar with these two biocides. In view of the results of this investigation, we recommend that further site-specific studies be conducted with OPO and TRC in estuarine water of the same quality before decisions can be made concerning the possible use of ozone in estuarine industrial or municipal wastwaters.

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